IN THE CLAIMS:

Please cancel claims 3, 6, 10, 11, 12, 17, 21 and 25.

Please amend claims 1, 2, 5, 7-9, 15, 18, 19, 22, and 26 as follows:

1. (Currently Amended) A process of preparing membrane vesicles from the culture supernatant of a biological sample, wherein said biological sample comprises membrane vesicles produced by antigen presenting cells that have been sensitized to one or more selected antigens, said method comprising at least

a filtration of the culture supernatant, followed by a tangential ultrafiltration to produce a biological sample enriched with membrane vesicles;

~~one~~ an anion exchange chromatography treatment performed under pressure of the enriched sample followed or preceded by gel permeation chromatography of said enriched sample; and

a sterilising filtration step.

2. (Currently Amended) Process according to claim 1, wherein said anion exchange chromatography is performed on a support functionalised with a quaternary amine.

3. (Cancelled)

4. Process according to claim 1, wherein the biological sample is selected from a biological fluid, a culture supernatant, a cell lysate and a pre-purified solution.

5. (Currently Amended) A process of preparing membrane vesicles from a biological sample, wherein said process comprises at least:

a) the culture of a population of membrane vesicles producing antigen presenting cells under conditions enabling the release of vesicles, wherein said antigen presenting cells have been sensitized to one or more selected antigens,

b) a ~~treatment~~ filtration of the culture supernatant of the ~~sample~~ cells, followed by a tangential ultrafiltration to prepare a sample enriched with membrane vesicles, ~~and~~

c) an anion exchange chromatography treatment performed under pressure ~~or~~ and a gel permeation chromatography treatment of the sample, and~~.~~

d) a sterilising filtration step of the sample.

6. (Cancelled).

7. (Currently Amended) Process according to claims 5 ~~or 6~~, wherein the enrichment step also comprises a clarification stage.

8. (Currently Amended) Process according to claim 5 ~~or 6~~, wherein the enrichment step comprises an affinity chromatography step.

9. (Currently Amended) Process according to claim 5 ~~or 6~~, characterised in that ~~wherein~~ the enrichment step comprises a ~~low-speed~~ centrifugation step realized at a speed below 1000g or a filtration.

10. (Cancelled)

11. (Cancelled)

12. (Cancelled)

13. Process according to claim 1, wherein the membrane vesicles have a diameter between approximately 60 and 90 nm.

14. Process according to claim 1, wherein the antigen presenting cells comprise dendritic cells, B lymphocytes, macrophages or mastocytes.

15. (Currently Amended) Process according to claim 5 ~~6~~, characterised ~~wherein~~ in that the membrane vesicles are vesicles produced by human dendritic cells.

16. (Cancelled)

17. (Cancelled)

18. (Currently Amended) A process of preparing membrane vesicles, characterised in that it~~wherein said process~~ comprises the following steps:

a) obtaining a population of immature dendritic cells sensitized to one or more selected antigen~~t~~s,

b) culturing the dendritic cells under conditions enabling the production of membrane vesicles,

c) treating the culture supernatant of said cells to produce a biological sample enriched with membrane vesicles by a filtration of the culture supernatant followed by a tangential ultrafiltration, ~~and~~

d) purifying the membrane vesicles using a process comprising at least an anion exchange chromatography treatment performed under pressure~~or~~ and a gel permeation chromatography ~~step~~ of the sample, and, ~~.~~

19. (Currently Amended) Process according to claim 18~~17~~, characterised in that~~wherein~~ the dendritic cells are obtained from a biological sample from a subject.

20. (Cancelled)

21. (Cancelled)

22. (Currently Amended) Process according to claim 18~~17~~, characterised in that~~wherein~~ during step b), the dendritic cells are cultured under conditions stimulating membrane vesicle production.

23. (Cancelled)

24. (Cancelled)

25. (Cancelled)

26. (Currently Amended) Process of preparing membrane vesicles from a biological sample, characterised in that it comprises~~ing~~:

a) the culture of a population of membrane vesicle producing tumoral cells under conditions enabling the release of vesicles,

b) a membrane vesicle enrichment step comprising a filtration followed by a tangential ultrafiltration, ~~and~~

c) an anion exchange chromatography treatment performed under pressure and a ~~or~~ gel permeation chromatography treatment of the sample, and~~.~~

d) a sterilising filtration step of the sample.

27. (Previously Presented) Process according to claim 26, wherein the tumoral cells are human tumoral cells.

RESPONSE

THE AMENDMENTS TO THE CLAIMS TRAVERSE ALL OF THE § 112 ISSUES.

Claim 2 is amended to correct a typographical error. Claim 3 is cancelled. Claim 9 is amended to replace the term "low" with a quantitative value. These amendments are believed to resolve all § 112 issues and the amendments are non-substantive on the merits of the invention.

THE ART-BASED REJECTIONS UNDER 35 U.S.C. § 102 ARE TRAVERSED – THE AMENDED CLAIMS RECITE ELEMENTS NOT DISCLOSED BY THE REFERENCES.

Numerous prior art references have been cited against the pending claims 1, 2, 3, 5, 7, 11, 12 and 23 of the application under 35 U.S.C. § 102(b) and 35 U.S.C. § 103(a). However, claims 1, 5, 18 and 26 have been amended and now specify that the process comprises a filtration of the culture supernatant, followed by a tangential ultrafiltration to produce a biological sample enriched with membrane vesicles, that the anion exchange chromatography is performed under pressure and is followed or preceded by gel permeation chromatography. Claim 9 is amended to specify that the centrifugation is realized at a speed below 1000 g.

Claims 7, 8, 15, 19 and 22 have also been adapted to the new wording and amendments of the claims.

These amendments find explicit support in the specification as filed (notably page 5, lines 13-18, page 15, lines 9-10 and page 16, lines 26-28), do not add any new matter, and entry thereof is respectfully requested. The following headings address each art-based rejection of the previous claims and correspond to the paragraphs of the Final Action in the parent case.

18. Claim 25 is Cancelled Without Prejudice. Amended Claims 26 and 27 Are Not Rendered Obvious by the Combination of Zitvogel and Dubinsky et al. and Gordon.

Claims 26 (as amended) and 27 read as follows:

26. (Currently Amended) Process of preparing membrane vesicles from a biological sample, characterised in that it comprises:

a) the culture of a population of membrane vesicle producing tumoral cells under conditions enabling the release of vesicles,

b) a membrane vesicle enrichment step comprising a filtration followed by a tangential ultrafiltration, ~~and~~

c) an anion exchange chromatography treatment performed under pressure and a ~~or~~ gel permeation chromatography treatment of the sample, and~~.~~

d) a sterilising filtration step of the sample.

27. (Previously Presented) Process according to claim 26, wherein the tumoral cells are human tumoral cells.

The Zitvogel et al. reference broadly relates to dexosomes, but does not describe the combination of botangential ultrafiltration and gel permeation chromatography steps, combined with a pressurized anion exchange chromatography. Dubinsky et al. disclose the preparation of apical membrane vesicles from bovine tracheal epthelium, but do not teach the combined chromatography and tangential filtration steps.

Thus, even the proposed combination of the references does not produce a tangential ultrafiltration to produce a biological sample enriched with membrane vesicles, an anion exchange chromatography treatment of the enriched sample performed under pressure, followed or preceded by gel permeation chromatography of said enriched sample, and, in the end, a sterilising filtration step.

Therefore, claims 26 and 27 are not rendered obvious by the combination of Zitvogel, Dubinsky and Gordon because the combination of references does not disclose each element of the pending claims and, therefore, cannot establish a prima facie case against the claims under § 103(a).

20. Amended Claims 5, 7, and 9 Are Not Rendered Obvious by the Combination of Raposo et al., Von der Decken, Gordon, Smith et al., Nishino et al., Tanaka et al., and Seeger.

Amended claim 5 reads as follows:

> 5. (Currently Amended) A process of preparing membrane vesicles from a biological sample, wherein said process comprises at least:
> a) the culture of a population of membrane vesicles producing antigen presenting cells under conditions enabling the release of vesicles, wherein said antigen presenting cells have been sensitized to one or more selected antigens,
> b) a ~~treatment~~ filtration of the culture supernatant of the ~~sample~~ cells, followed by a tangential ultrafiltration to prepare a sample enriched with membrane vesicles, ~~and~~
> c) an anion exchange chromatography treatment performed under pressure ~~or~~ and a gel permeation chromatography treatment of the sample, and~~.~~
> d) a sterilising filtration step of the sample.

Claim 5 requires that the process comprises a filtration of the culture supernatant of the cells, followed by a tangential ultrafiltration to prepare a sample enriched with membrane vesicles, that the anion exchange chromatography is performed under pressure and is followed or preceded by gel permeation chromatography and also comprise a further sterilising filtration step of the treated preparation. The core reference in this § 103 combination rejection, Raposo et al., does not teach anion exchange chromatography and does not teach the production of membrane vesicles from antigen producing cells. Furthermore, in Von der Decken, microsomes are prepared from pellets consisting of ribosomes derived from homogenized liver -- none of the method steps recited as the elements of claim 5 are disclosed. Gordon, Smith, Tanaka, Nishino, and Seeger do not describe the

the origin of the membrane shed vesicles mentioned in the reference. Accordingly, these cited references do not, either individually or collectively, suggest that membrane vesicles are produced by antigen presenting cells and do not meet the other limitations of the claims. Thus, even in combination, the references do not teach several elements of the amended claims and cannot form the basis for a prima facie case of obviousness under § 103.

22. Claims 1-7, 9, 13-15, 17-19, 21, 22, and 25 Are Not Rendered Obvious by the Combination of Zitvogel et al., Thiery et al., Vaandrager et al., and Chen et al.

As noted above, Zitvogel relates to dexosomes but does not teach anion exchange chromatography. The same can be said of Thiery et al. which does not teach the production of membrane vesicles from antigen presenting cells. Vaandrager et al. merely describes vesicles derived from villi cells, but does not disclose the remaining elements of the amended claims. Chen et al. teach that polyunsaturated acid-containing molecular species of phosphatidylserine can be isolated from bovine extract by means of anion exchange chromatography, but do not suggest the possibility of purifying vesicles produced by antigen presenting cells by such a chromatography step. Thus, even in combination, these references do not disclose each element of the pending claims and cannot render the claims obvious under § 103(a).

23. Claims 1-4, 5, 6-9, 11, 13-15, 17-19, 21, 22, and 25 Are Not Anticipated by the Combination of Zitvogel et al., Thiery et al., Chen et al., the Zitvogel et al. Nature Medicine Publication, Amigorena, and Ogle et al.

The infirmity in the Zitvogel et al., Thiery et al., Vaandrager et al. and Chen et al. references are described above. Ogle et al. teach affinity chromatography, but do not apply this technology to vesicles produced by antigen presenting cells. None of the references provide the steps of filtration of the culture supernatant, followed by tangential ultrafiltration to produce biological samples

enriched with membrane vesicles, anion exchange chromatography performed under pressure, together with gel permeation chromatography, and a concluding sterilising filtration step. Any combination of the cited references fails to teach these elements, when considered as part of the invention as a whole, such as to render the claimed subject matter obvious under § 103(a).

24. Claims 1-15, 7-19, 21, 22, and 25 Are Not Rendered Obvious by the Combination of Zitvogel et al., Thiery et al., Vaandrager et al., Chen et al., Amigorena, Ogle et al., and Lenk et al.

The addition of the Lenk et al. reference does not cure the infirmity of the disclosure of the above cited references, either alone or in combination. Although Lenk et al. teach a sterile filtration as applied to liposomes, Lenk et al. do not contemplate applying the technique to vesicles produced by antigen presenting cells.

25. No Combination of The References of Record Establish a Prime Facie Case and the Requisite Rationale Does Not Exist to Reject the Amendment Claims Under § 103(a)

While Applicants appreciate the Examiner's thorough review of the references, and without acquiescence of the rational cited by the Examiner for the motivation to combine the references, the amended claims recite a combination not disclosed by any legitimate combination of the refernces and describe subject matter where no motivation by one of ordinary skill in the art can be cited to yield the presently claimed subject matter. None of the identified prior art, either alone or in combination, discloses or suggests a process of preparing membrane vesicles from the culture supernatant of a biological sample, wherein said biological sample comprises membranes vesicles produced by antigen presenting cells that have been sensitized to one or more selected antigens, said method comprising at least a filtration of the culture supernatant, followed by a tangential ultrafiltration to produce a biological sample enriched with membrane vesicles, an anion exchange

chromatography treatment step performed under pressure, followed or preceded by gel permeation chromatography of said enriched sample, and, in the end, a sterilising filtration step.

Applicants respectfully note that to establish a *prima facia* case of obviousness, all elements of the claimed invention must be shown in a combination of the prior art and some motivation to combine these elements in the claimed manner must be provided by the Examiner based on a rationale that is explicitly or inherently demonstrated in the art. Applicants submit that such motivation cannot be demonstrated here for the amended claims because the references do not fairly disclose each element of the claims when taken as a whole, even if isolated reference to individual elements is present.

THE PRESENT CLAIMED INVENTION PRODUCES ADVANTAGES NOT CONTEMPLATED BY THE REFERENCES EITHER ALONE OR IN COMBINATION.

Applicants wish to stress that the present invention advantageously allows the high purification of membrane vesicles, particularly of vesicles which include heterologous molecules (antigenic determinants or epitopes for example) without damaging said heterologous molecules. It is particularly striking and unexpected that the integrity and functionality of complex and sensitive molecular complexes such as MHC-peptides complexes, can be preserved in such chromatographic techniques. Purified vesicles of the invention thus retain their biological properties, i.e. their ability to present their heterologous molecules or transmit them to antigen presenting cells.

The different steps of the processes are organized to separate the membrane vesicles from potential biological contaminants. According to the description (page 4), the claimed process "*allows advantageously the purification of membrane vesicles under conditions compatible with an industrial use and pharmacological applications*". The "*processes of the invention may be applied*

both for individualized autologous exosome preparations obtained from established cell lines, for experimental or biological use or prophylactic or therapeutic vaccination purposes" (see also page 18, lines 4-9).

As indicated on page 40, lines 15-24, "between 99 and 99.5% of the proteins in the culture supernatant are eliminated, while retaining the exosomes. The process preserves the integrity of the exosomes in electron microscopy. Therefore, this process is specific for exosome purification." None of the cited references discloses or suggests a combination of treatments as presently claimed, which is thus inventive.

For these reasons, Applicants submit that the amended claims are in condition for allowance and request such action accordingly. If the Examiner has any questions regarding the foregoing, or if the Examiner believes that an interview would facilitate the examination of this application, or if any additional information is required, the Examiner is invited to contact the undersigned at 949/567-6700, X 7740.

Respectfully submitted,

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